Journal of Diabetes and Its Complications 29 (2015) 1203–1210

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Retrospective analysis of safety and efficacy of liraglutide monotherapy and sulfonylurea-combination therapy in Japanese type 2 diabetes: Association of remaining β-cell function and achievement of HbA1c target one year after initiation

Ryota Usui [a,1], Daisuke Yabe [a,b,c,d,*], Hitoshi Kuwata [a,c], Kenta Murotani [e], Takeshi Kurose [a,c], Yutaka Seino [a,c,*]

[a] Center for Diabetes, Endocrinology, and Metabolism, Kansai Electric Power Hospital, 2-1-7 Fukushima-ku, Osaka, 553-0003, Japan
[b] Center for Metabolism and Clinical Nutrition, Kansai Electric Power Hospital, 2-1-7 Fukushima-ku, Osaka, 553-0003, Japan
[c] Yutaka Seino Distinguished Center for Diabetes Research, Kansai Electric Power Medical Research Institute, 2-1-7 Fukushima-ku, Osaka, 553-0003, Japan
[d] Division of Molecular and Metabolic Medicine, Department of Physiology and Cell Biology, Kobe University Graduate School of Medicine, 1-5-6 Minatojimaminamimachi, Chuo-ku, Kobe, 650-0047, Japan
[e] Center for Clinical Research, Aichi University Hospital, 1-1 Yazakokarimata, Nagakute, Aichi, 480-1195, Japan

ARTICLE INFO

Article history:
Received 18 May 2015
Received in revised form 16 July 2015
Accepted 18 July 2015
Available online 21 July 2015

Keywords:
Type 2 diabetes
GLP-1 receptor agonist
β-Cell function
Glucagon stimulation test
C-peptide

ABSTRACT

Aims: The GLP-1 receptor agonist liraglutide improves impaired pancreatic β-cell function, thereby exerting glucose-lowering effects. However, the association of remaining β-cell function with long-term therapeutic efficacy of liraglutide remains largely unknown.
Methods: Patients with type 2 diabetes who started liraglutide as monotherapy or sulfonylurea-combination therapy were retrospectively analyzed to identify possible associations of indices related to β-cell function including increments of C-peptide immunoreactivity in glucagon stimulation test (GST-ΔCPR) with achievement of HbA1c <7.0% at 54 weeks after liraglutide initiation.
Results: Among 165 subjects continuing liraglutide for 54 weeks, 66 received additional oral anti-diabetic drugs (OADs) during the period. Of those continuing liraglutide without receiving additional OADs, 41 subjects achieved HbA1c <7.0% at 54 weeks, while 49 subjects did not. Subjects achieving HbA1c <7.0% showed higher values of GST-ΔCPR. Receiver-operating analysis revealed 2.34 ng/mL as the cut-off value for HbA1c <7.0% achievement in these subjects. Subjects with GST-ΔCPR >2.34 ng/mL showed continuous and substantial HbA1c reduction throughout the 54 weeks. In Kaplan–Meier analysis, subjects with GST-ΔCPR >2.34 ng/mL showed longer therapeutic durability of initial liraglutide therapy with no additional OADs or insulin.
Conclusions: Despite numerous limitations, these results indicate that long-term efficacy of liraglutide is associated with remaining β-cell function at initiation.

Funding: Grant-in-Aid for Young Scientists (B) from Japan Society for Promotion of Science and Grants for young researchers from Japan Association for Diabetes Education and Care to DY; Grants from Japan Vascular Disease Research Foundation to YS.

Duality of interest: D.Y. received consulting and/or speaker fees from Eli Lilly, Merck, Sharp and Dohme, Sanofi, Novo Nordisk, Boehringer Ingelheim, Takeda and Taisho pharmaceutical. D.Y. received clinical commissioned/joint research grants from Boehringer Ingelheim, and Eli Lilly. K.T. received consulting and/or speaker fees from Astellas, Boehringer Ingelheim, Sanofi, Novo Nordisk, Sanofi, Novo Nordisk, Merck, Sharp and Dohme, Takeda, Kowa, Astellas, Tanabe Mitsubishi, Kaken Pharm, AstraZeneca, Daiichi-Sankyo, Kyowa Kirin. K.T. also received clinical commissioned/joint research grants from Boehringer Ingelheim, Novo Nordisk, Merck, Sharp and Dohme, Takeda, Ono Pharm, Eli Lilly, Teijin, Sanofi. Y.S. received consulting and/or speaker fees from Eli Lilly, Sanofi, Novo Nordisk, Glaxo-Smith-Kline, Taisho pharmaceutical, Astellas Pharma, BD, Boehringer Ingelheim, Johnson & Johnson and Takeda. Y.S. received clinical commissioned/joint research grants from Boehringer Ingelheim, Eli Lilly. R.Y.., H.K., and K.M. report no conflict of interest relevant to this study.

Contribution statement: RU, DY and YS take responsibility for the contents of the article. RU, DY and YS designed the research; collected data, and analyzed data and wrote the manuscript. H.K. and K.T. contributed to data collection, and discussion. KM contributed to statistical analysis, and discussion.

* Corresponding authors at: Center for Diabetes, Endocrinology, and Metabolism, Kansai Electric Power Hospital, 2-1-7 Fukushima-ku, Osaka, 553-0003, Japan. Tel.: +81 6 6458 5821; fax: +81 6 6458 6994.
E-mail addresses: ydaisuke-kyoto@umin.ac.jp (D. Yabe), seino.yutaka@e2.kepco.co.jp (Y. Seino).
[1] Present address: Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University, 54 Shogo-in Kawahara-cho, Sakyo-ku, Kyoto, 606-8507, Japan.

http://dx.doi.org/10.1016/j.jdiacomp.2015.07.020

1. Introduction

Type 2 diabetes is a heterogeneous disease characterized by β-cell dysfunction and insulin resistance (DeFronzo, 1988; Stumvoll, Goldstein, & van Haeften, 2005; Yabe, Seino, Fukushima, & Seino, 2015). Due to progressive decline in β-cell function, anti-diabetic treatment regimens must be adjusted over time based on estimates of the remaining insulin secretory capacity. Conventional anti-diabetic drugs that compensate for reduced insulin secretion include insulin and sulfonylurea (SU), both of which have been shown to maintain optimal glycemic control and to prevent progression of diabetes-related complications (Anonymous, 1999; Holman, Paul, Bethel, Matthews, & Neil, 2008). However, insulin and SU are associated with varying degrees of hypoglycemia and weight gain (Anonymous, 1998; Best et al., 2012). Glucagon-like peptide-1 (GLP-1) receptor agonists are emerging anti-diabetic drugs that enhance insulin secretion glucose-dependently as well as suppress glucagon secretion and slow gastric emptying, thereby improving glycemic control and reducing bodyweight in patients with type 2 diabetes (Meier, 2012; Yabe, Kuwata, Usui, Kurose, & Seino, 2015; Yabe & Seino, 2014). Clinical trials of GLP-1 receptor agonists comparing their efficacy and safety with those of insulin or SU show that GLP-1 receptor agonists are capable of achieving appropriate glycemic control with reduced risk of hypoglycemia and bodyweight gain (Meier, 2012; Yabe, Kuwata, Usui, Kurose, & Seino, 2015; Yabe & Seino, 2014). However, the long-term therapeutic effects of GLP-1 receptor agonists in clinical practice need to be evaluated.

Liraglutide is a once daily injectable GLP-1 receptor agonist, with a Lys34Arg substitution and the addition of a C16-fatty acid at Lys26 in human GLP-1 (Best et al., 2012; Faber & Binder, 1977; Fujita et al., 2015; Funakoshi et al., 2011; Gjessing, Matzen, Froland, & Faber, 1987; Greenbaum et al., 2008; Hendriksen, Faber, Drejer, & Binder, 1977; Iwao, Sakai, & Sata, 2013; Iwata et al., 2014; Kajinuma et al., 1979; Kaku, Rasmussen, Clauson, & Seino, 2010; Kaku, Rasmussen, Nishida, & Seino, 2011; Kondo et al., 2013; Kozawa et al., 2012; Lapolla et al., 2015; Matsuda, Kamata, Iwamoto, Sakamoto, & Kuzuya, 1985; Meier, 2012; Ponzani, 2013; Retnakaran, Kramer, Choi, Swaminathan, & Zinman, 2014; Saisho et al., 2011; Seino, Rasmussen, Clauson, & Kaku, 2012; Seino, Rasmussen, Nishida, & Kaku, 2010; Seino et al., 2010; Shao, Yuan, Feng, Zhang, & Guo, 2014; Toyoda, Yokoyama, Abe, Nakamura, & Suzuki, 2014; Usui et al., 2013; Vilsboll et al., 2008; Yabe, Kuwata, Usui, Kurose, & Seino, 2015; Yabe & Seino, 2011, 2014; Yamada et al., 2006). This endows resistance to degradation of GLP-1 mediated by dipeptidyl peptidase-4 and its stabilization in human circulation as an albumin bound form. Liraglutide has been shown to exert its glucose-lowering effects partly by ameliorating impaired glucose-dependent insulin secretion from β-cells, one of the major defects in type 2 diabetes (Seino et al., 2012; Vilsboll et al., 2008). It has been reported that early liraglutide initiation in type 2 diabetes as well as in experimental animals exerts superior improvement on β-cell function and better glycemic control (Retnakaran et al., 2014; Shao et al., 2014), suggesting that remaining β-cell function might predict the glucose-lowering effects of liraglutide. While several clinical studies have demonstrated that liraglutide results in greater HbA1c reduction in early stage diabetes (Iwao et al., 2013; Lapolla et al., 2015; Ponzani, 2013; Toyoda et al., 2014), clinical investigations of direct association of β-cell function with glucose-lowering effects of liraglutide, especially at longer time points, are few. To assess β-cell function in patients with type 2 diabetes in clinical settings, several indices using serum C-peptide immunoreactivity (CPR) are used: glucagon-stimulated increments of CPR (Faber & Binder, 1977; Fujita et al., 2015; Gjessing et al., 1987; Hendriksen et al., 1977), fasting and postprandial C-peptide index (CPI) (Funakoshi et al., 2011; Saisho et al., 2011), and secretory units of islets in transplantation (SUIT) (Funakoshi et al., 2011; Iwata et al., 2014; Yamada et al., 2006). While glucagon-stimulated increments of CPR are ideal for assessment of β-cell function, CPI and SUIT were used because they are more readily evaluated in actual clinical settings. These CPR-related indices for β-cell function have been used to predict requirements for insulin injections in patients with type 2 diabetes (Funakoshi et al., 2011; Iwata et al., 2014; Saisho et al., 2011). We and others have reported that these CPR-related indices can predict successful switch from insulin to liraglutide (Kondo et al., 2013; Kozawa et al., 2012; Usui et al., 2013), but the associations of these CPR-related indices with long-term therapeutic efficacy of liraglutide have not been investigated.

In the current study, we evaluated the role of remaining β-cell function in relation to long-term therapeutic efficacy of liraglutide in Japanese patients with type 2 diabetes.

2. Materials and methods

2.1. Participants

Two hundred seventy-two patients with type 2 diabetes who started liraglutide therapy at Kansai Electric Power Hospital between June 2010 and March 2012 were retrospectively analyzed in the current study. None of the patients had type 1 diabetes, pancreatic disease, liver disease, renal disease, malignancy or were taking diabetogenic medication or were pregnant. Type 1 diabetes was diagnosed by auto-antibodies such as anti-GAD antibody and anti-IA-2 antibody according to the diagnostic criteria of type 1 diabetes by the Japan Diabetes Society (Seino, Nanjo, et al., 2010). Patients with renal failure and/or those taking dialysis were excluded, since serum CPR levels are modified in these conditions. Insulin-dependent patients were also excluded for lack of β-cell function. Insulin-dependency was defined by combinations of fasting CPR ≤0.5 ng/mL, stimulated CPR ≤1.0 ng/mL, and urinary CPR ≤20 μg/day (Matsuda et al., 1985). The duration of type 2 diabetes was defined as years after diagnosis of the disease according to the criteria of the Japan Diabetes Society. Physical and laboratory data including HbA1c were acquired in all patients before and every 6 weeks after starting liraglutide therapy. Inclusion and exclusion of the study subjects are summarized in Fig. 1. Of the 272 patients, 22 were excluded from analysis because they were referred to other clinics or stopped hospital visits for unknown reasons during the 54 week period. The baseline clinical profiles and parameters of the remaining 250 patients are shown in Table 1. Of them, 26 patients discontinued liraglutide during the 54 weeks due to adverse events (nausea n = 6, skin rash n = 1, others n = 19), and 59 patients discontinued liraglutide due to hyperglycemia. Sixty-six patients received additional OADs during the 54 weeks (8 patients, HbA1c at 54 weeks was not available), and the remaining 99 patients continued liraglutide during the 54 weeks without adding any OAD (9 patients, HbA1c at 54 weeks was not available). Severe hypoglycemia was defined as requiring assistance of another person to actively administer carbohydrate, glucagon or other resuscitative actions. Non-severe hypoglycemia was not evaluated in the current study, since its definition varied among the physicians in charge.

2.2. Measurements

HbA1c was measured using high performance liquid chromatography with cation-exchange resins that separate the stable form of β-N1-mono-deoxyfructosyl Hb; the values are shown in National Glycohemoglobin Standardization Program units as recommended by the Japan Diabetes Society (Seino, Nanjo, et al., 2010). Glucagon stimulation test (GST) was carried out after an overnight fast by measuring serum CPR at fasting or 6 min after intravenous injection of 1 mg glucagon (CPR-0 min and CPR-6 min, respectively) (Kajinuma et al., 1979). In patients who received insulin therapy before starting liraglutide therapy, insulin injections were continued to avoid hyperglycemia until the night before measuring fasting and/or

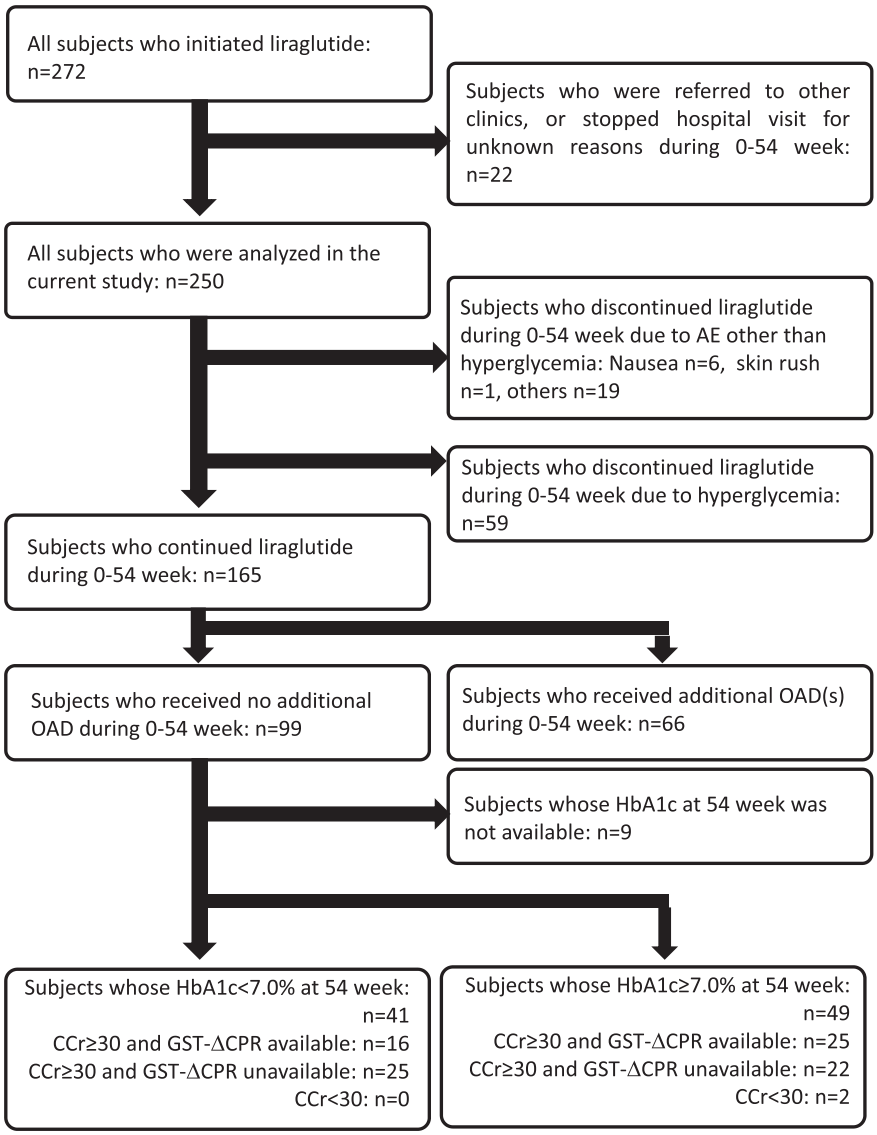


Fig. 1. Schematic diagram of the current retrospective analysis.

glucagon-stimulated levels of serum C-peptide in the morning, and were stopped until the end of the glucagon stimulation test. Antidiabetic drugs were also stopped for the glucagon test, but were maintained until 1 day before the test. The glucagon stimulation test was carried out when fasting plasma glucose levels reached ≥80 mg/dL similarly to previous studies (Greenbaum et al., 2008) because hypoglycemia might affect β-cell response to glucagon. Increments of CPR after glucagon stimulation test (GST-ΔCPR), C-peptide index (CPI) and secretory unit of islet in transplantation (SUIT) were calculated as follows: GST-ΔCPR (ng/mL), (CPR-6 min) − (CPR-0 min); CPI, (fasting CPR)/(fasting plasma glucose) × 100; SUIT, 1500 × (fasting C-peptide)/((fasting plasma glucose) − 63). Serum C-peptide was measured using lumipulse presto C-peptide (Fujirebio Inc., Tokyo, Japan). Intra- and inter-assay variations of measurement of serum CPR levels were 1.96%–2.97% and 1.06%–2.60%, respectively. Other laboratory measurements including plasma glucose were measured by standard assays.

2.3. Statistical analysis

Patient characteristics and results are reported as mean ± standard error of the mean unless otherwise stated. Statistical analysis was carried out using IBM SPSS Statistics version 22 (SAS Institute Inc., Cary, NC, USA). Repeated measures were analyzed by mixed effects model. Clinical parameters between the two groups at single time-points were compared by Mann–Whitney U-test. The receiver-operating characteristic (ROC) curve was constructed for CPR-0 min, CPR-6 min, GST-ΔCPR, CPI and SUIT. Sensitivity, specificity, cut-off point, area under the ROC curve (AUC) and the likelihood ratio were also calculated. The Kaplan–Meier method was used to analyze durability of initial therapy, and the difference was examined using log-rank test. p values <0.05 were considered statistically significant.

3. Results

Of 165 patients who continued liraglutide during the 54 weeks, 66 patients also received additional OADs during the test period (Fig. 1). The remaining 99 patients continued liraglutide without receiving additional OADs. Of these patients, 41 achieved HbA1c less than 7.0% at 54 weeks. Sixteen of these patients had GST data at baseline; 25 of them did not take the GST. Forty-nine of the patients showed HbA1c 7.0% or more at 54 weeks. Twenty-five of these patients had GST data; 22 of them did not take the GST, and 2 of them were excluded from analysis because their Ccr level was less than 30, which might possibly affect GST values. Nine of the 99 patients had no HbA1c data at

Table 1
Baseline characteristics of patients with type 2 diabetes before liraglutide initiation.

	All patients	Patients receiving no additional OAD during 0–54 weeks	Patients receiving additional OAD during 0–54 weeks
N (% male)	250 (66.8%)	90 (66.7%)	58 (62.1%)
Age at 0 week (years)	63.0 ± 0.8	62.0 ± 1.2	62.7 ± 1.6
Estimated duration at 0 week (years)	13.6 ± 0.6	11.6 ± 0.9	12.6 ± 1.1
BMI at 0 week (kg/m^2)	26.5 ± 0.3	27.0 ± 0.5	26.3 ± 0.7
Initiated as monotherapy or sulfonylurea combination (%)	60.0/40.0	57.8/42.2	72.4/27.6
Insulin use before liraglutide initiation (%)	80	74	81
OAD use before liraglutide initiation (%)			
Sulfonylureas	28	30	29
Metformin	24	21	26
Glinides	13	13	12
α-glycosidase inhibitors	10	7	12
Thiazolidinedione	6	1	7
DPP-4 inhibitors	8	6	9
CPI at 0 week	1.28 ± 0.06	1.41 ± 0.11	1.25 ± 0.13
SUIT at 0 week	46.4 ± 3.4	48.2 ± 4.4	41.6 ± 4.3
GST-CPR0 min at 0 week	1.64 ± 0.09	1.87 ± 0.18	1.67 ± 0.20
GST-CPR6 min at 0 week	3.27 ± 0.18	4.01 ± 0.36	3.18 ± 0.35
GST-ΔCPR at 0 week	1.63 ± 0.10	2.14 ± 0.20	1.52 ± 0.19
Liraglutide dosage at 54 weeks (mg)	–	0.79 ± 0.02	0.85 ± 0.01
HbA1c at 0 week (%)	8.1 ± 0.1	7.8 ± 0.1	8.2 ± 0.2*
HbA1c at 54 weeks (%)[a]	7.4 ± 0.1	7.1 ± 0.1	7.9 ± 0.1*
Patients with HbA1c <7% at 0 week (%)	16.0	22.2	12.1
Patients with HbA1c <7% at 54 weeks (%)[a]	32.8	45.6	13.8

Each value represents the mean ± SEM. BMI, body mass index. CPI, 100 × [fasting serum C-peptide (ng/mL)]/[fasting plasma glucose (mg/dL)]. GST-ΔCPR, increment of serum C-peptide levels before and 6 min after iv administration of 1 mg glucagon (GST-CPR0 min and GST-CPR6 min, respectively). BMI, body mass index; CPI, C-peptide index; DPP-4, dipeptidyl peptidase-4; GST, glucagon stimulation test; OAD, oral anti-diabetic drugs; SUIT, secretory unit of islet in transplantation. Liraglutide dosages at 54 weeks for patients receiving no additional OAD during 0–54 weeks and those receiving additional OAD during 0–54 weeks are shown. Liraglutide up to 0.9 mg q.d. is approved in Japan.

[a] Values are calculated for patients who have HbA1c values at 54 weeks.

* Denotes $p < 0.05$ in Mann–Whitney U-test (*versus* continued).

54 weeks. Baseline characteristics of the patients are shown in Table 1. Compared with patients receiving additional OADs during 0–54 weeks, patients receiving no additional OAD showed significantly lower HbA1c at 0 week (7.8% ± 0.1% vs 8.2% ± 0.2%) and 54 weeks (7.1% ± 0.1% vs 7.9% ± 0.1%). The indices related to β-cell function showed a significantly higher value of GST-ΔCPR (ng/mL) (2.14 ± 0.20 *vs* 1.52 ± 0.19, $p < 0.05$), indicating that remaining β-cell function plays a role in the glucose-lowering effects of liraglutide.

To address possible correlation of remaining β-cell function with the glucose-lowering effects of liraglutide, indices related to β-cell function at baseline between patients with HbA1c <7.0% and those with HbA1c ≥7.0% at 54 weeks were compared. Patients having HbA1c <7.0% at 54 weeks showed significantly higher values of GST-CPR0 min, GST-CPR6 min, and GST-ΔCPR than those with HbA1c ≥7.0% at 54 weeks [GST-CPR0 min (ng/mL), 2.19 ± 0.25 vs 1.67 ± 0.24, $p = 0.049$; GST-CPR6 min (ng/mL), 5.03 ± 0.52 vs 3.36 ± 0.44, $p = 0.006$; GST-ΔCPR (ng/mL) 2.84 ± 0.33 vs 1.70 ± 0.22, $p = 0.003$] (Fig. 2A). They also had higher values of CPI and SUIT [CPI, 1.58 ± 0.19 vs 1.13 ± 0.12, $p = 0.065$; SUIT, 45.9 ± 6.4 vs 42.1 ± 6.3, $p = 0.331$], although the difference did not reach statistical significance. The mean values of GST-ΔCPR, CPI and SUIT in our patients were somewhat lower than those in healthy controls: GST-ΔCPR, 3.29 ± 1.21 ng/mL (Fujita et al., 2015) and 3.30 ± 1.14 ng/mL (Matsuda et al., 1985); CPI, 1.66 ± 0.55 (Fujita et al., 2015); and SUIT 100 ± 11.7 (Yamada et al., 2006).

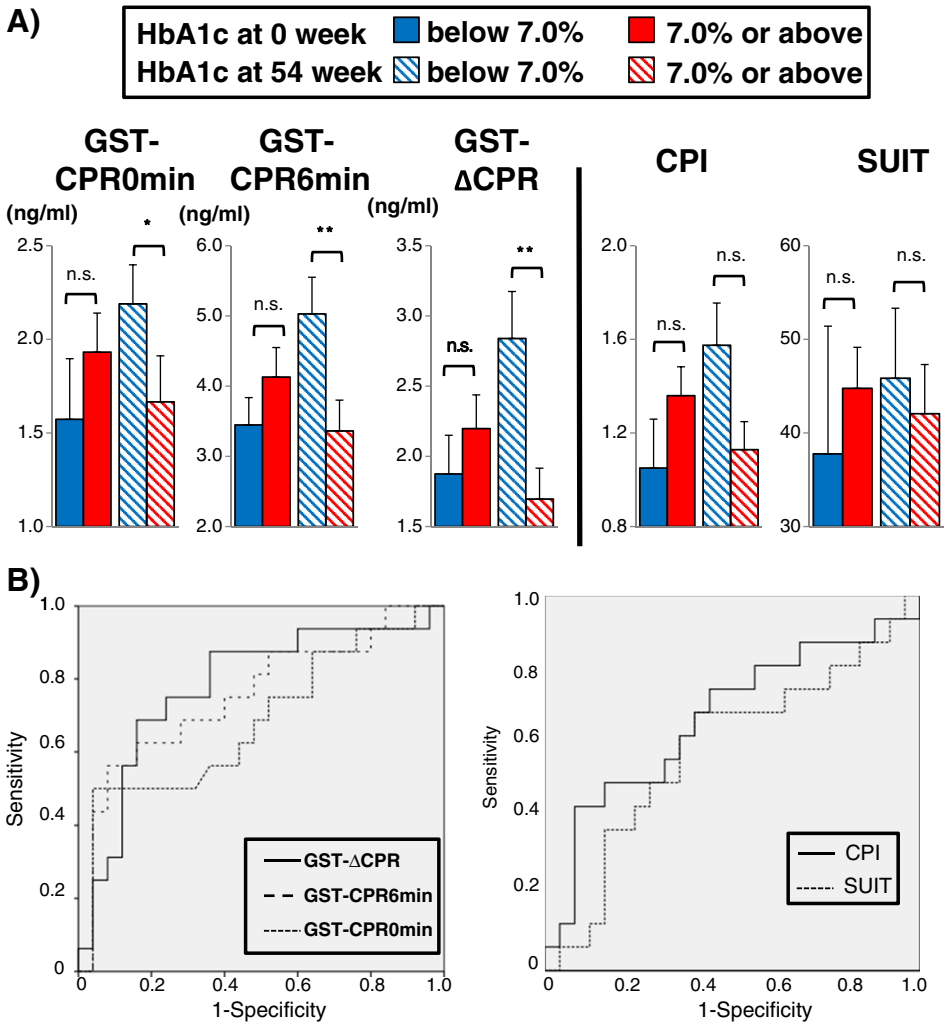
To determine the cut-off values for achieving HbA1c <7.0% at 54 weeks, ROC analyses were carried out for CPR-0 min, CPR-6 min, GST-ΔCPR, CPI and SUIT; GST-ΔCPR at baseline showed the largest AUC [0.780, 95% confidence interval 0.627–0.933] with the cut-off point estimated to be 2.34 ng/mL with 69% sensitivity and 84% specificity (Fig. 2B). AUCs and the cut-off points of other parameters such as GST-CPR0 min, GST-CPR6 min, CPI and SUIT are shown in Fig. 2B.

Using the estimated cut-off value of GST-ΔCPR at baseline, HbA1c and bodyweight over the 54 week period were compared between patients with GST-ΔCPR ≤2.34 ng/mL and those with GST-ΔCPR >2.34 ng/mL. In both groups, liraglutide initiation significantly reduced HbA1c throughout the 54 week period, but more so in patients with GST-ΔCPR >2.34 ng/mL. HbA1c changes between 0 and 54 weeks were significantly different in the two groups (GST-ΔCPR >2.34 ng/mL, −1.77% ± 0.57% and GST-ΔCPR ≤2.34 ng/mL, −0.66% ± 0.34%, $p < 0.01$) (Fig. 3A). Patients with GST-ΔCPR >2.34 ng/mL showed significantly higher bodyweight at baseline than those with GST-ΔCPR ≤2.34 ng/mL, but bodyweight was reduced by liraglutide initiation in both groups and bodyweight changes were not significantly different (GST-ΔCPR >2.34 ng/mL, −4.30 ± 0.93 kg and GST-ΔCPR ≤2.34 ng/mL, −4.45 ± 0.82 kg; $p = 0.507$) (Fig. 3B). Durability of liraglutide was assessed by Kaplan–Meier analysis of patient GST-ΔCPR at baseline. Patients who discontinued liraglutide due to AEs other than hyperglycemia and those who were referred to other clinics or stopped hospital visits for unknown reasons were excluded. Among the remaining patients (GST-ΔCPR ≤2.34 ng/mL, $n = 93$; and GST-ΔCPR >2.34 ng/mL, $n = 22$), time before receiving another OAD or switch to insulin to maintain glycemic control was significantly different between the two groups (log-rank test, $p = 0.0013$) (Fig. 4), indicating that liraglutide exerts longer-term therapeutic durability in patients with greater remaining β-cell function.

4. Discussion

In the current retrospective study, we find that the GLP-1 receptor agonist liraglutide has substantial and continuous HbA1c-lowering effects in patients with sufficient remaining pancreatic β-cell function. ROC analysis reveals cut-off values of GST-ΔCPR that predict HbA1c <7.0% achievement and associate with longer duration before patients are switched to insulin injection or additional OAD.

Consistent with previously reported clinical trials conducted in Japan (Kaku et al., 2010; Kaku et al., 2011; Seino, Rasmussen, Nishida, & Kaku, 2010; Seino, Rasmussen, Nishida, & Kaku, 2011), we found that liraglutide, initiated as monotherapy or SU-combination therapy,



	GST-CPR0min	GST-CPR6min	GST-ΔCPR	CPI	SUIT
AUC	0.684	0.755	0.780	0.672	0.590
95%CI	0.508-0.860	0.595-0.915	0.627-0.933	0.495-0.850	0.405-0.775
Cut-off point	2.47	4.35	2.34	1.86	35.0
Sensitivity (%)	50	63	69	44	69
Specificity (%)	96	80	84	92	60

Fig. 2. (A) Comparison of β-cell function related indices in patients with HbA1c less than 7% at 0 week (blue, n = 7; sulfonylureas-combination n = 3 and monotherapy n = 4) and 54 weeks (hatched blue, n = 16; sulfonylureas-combination n = 4 and monotherapy n = 12) and those with HbA1c 7% or above at 0 week (red, n = 34; sulfonylureas-combination n = 11, and monotherapy n = 23) and 54 weeks (hatched red, n = 25; sulfonylureas-combination n = 10, and monotherapy n = 15). GST-ΔCPR, increment of serum C-peptide levels before and 6 min after iv administration of 1 mg glucagon (GST-CPR0 min and GST-CPR6 min, respectively); CPI, 100 × [fasting serum C-peptide (ng/mL)]/[fasting plasma glucose (mg/dL)]; SUIT, 1500 × [fasting C-peptide (ng/ml)]/([fasting plasma glucose (mg/dl)] − 63). Each value represents mean ± SEM. * and ** denote $p < 0.05$ and $p < 0.01$, respectively (Mann–Whitney U-test, *versus* continued). (B) Receiver-operating characteristic (ROC) curves of β-cell function-related indices (CPR-0 min, CPR-6 min, GST-ΔCPR, CPI and SUIT) to predict for HbA1c less than 7% achievement at 54 weeks without adding additional anti-diabetic drugs. CPI, C-peptide index; CPR, C-peptide immunoreactivity; GST, glucagon stimulation test; SUIT, secretory unit of islet in transplantation.

improved HbA1c and reduced bodyweight substantially, with limited cases of adverse events in clinical settings. However, due to inadequate glycemic control, 23.6% of patients were discontinued on liraglutide and received insulin injection (Fig. 1) and 26.4% received additional OADs within 54 weeks after liraglutide initiation. Even among patients who continued liraglutide for 54 weeks without receiving additional OADs, only half achieved HbA1c < 7.0% (Fig. S1). We found that patients with shorter duration of type 2 diabetes and those not receiving insulin before liraglutide showed greater reduction of HbA1c independent of baseline BMI (Fig. S3). Others have reported that liraglutide exerts greater HbA1c-lowering effects with shorter duration of disease (Toyoda et al., 2014). Previously, we reported that increment of CPR in glucagon stimulation test predicts inadequate glycemic control within 12 weeks after insulin-to-liraglutide switch (Usui et al., 2013); others have reported that remaining β-cell function predicted short-term efficacy of liraglutide (Meier, Vilsbøll, Donsmark, Hartvig, & M.A. N., 2011). Our current study clearly indicates that liraglutide has sustained HbA1c-lowering effects in patients with greater β-cell function in the values of GST-ΔCPR (Fig. 2). ROC analysis reveals GST-ΔCPR 2.34 ng/mL as cut-off value for achieving HbA1c <7.0% at 54 weeks. This predicted both substantial HbA1c-lowering effects and longer time before switch to insulin injection or additional OAD. (Fig. 4) Our cut-off values for achieving HbA1c <7.0% (GST-ΔCPR 2.34 ng/mL and CPI 1.86) are considerably higher than those reported to avoid hyperglycemia within 12 weeks after insulin-to-liraglutide switch (Kozawa et

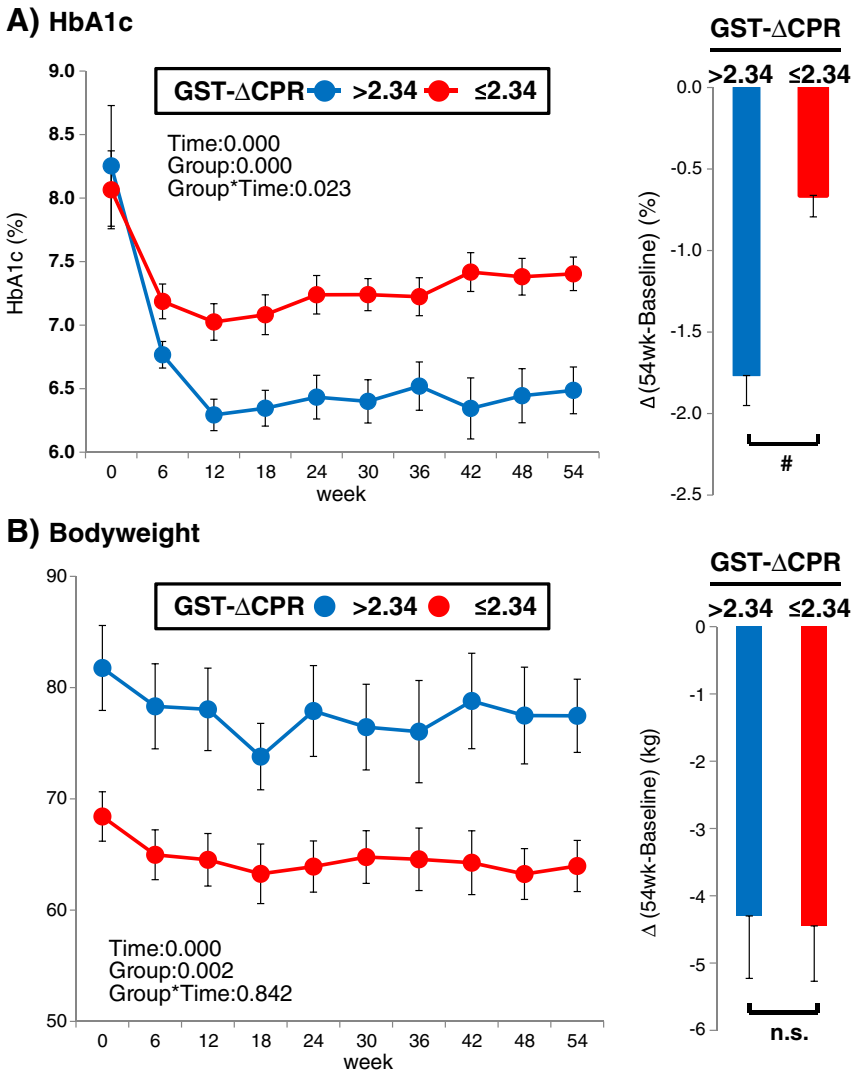


Fig. 3. Changes during 54 week observational periods in HbA1c (A) and bodyweight (B) in patients with GST-ΔCPR >2.34 ng/mL (n = 26; sulfonylureas-combination n = 13 and monotherapy n = 13) and those with GST-ΔCPR ≤2.34 ng/mL (n = 15; sulfonylureas-combination n = 1 and monotherapy n = 14). Each value represents mean ± SEM. Time course curves were analyzed by mixed effects models including group, time and the interaction of group and time, and p values are indicated.

al., 2012; Usui et al., 2013) and for HbA1c <7.0% at 24 weeks (Kondo et al., 2013). This may be due to 1) our longer observational period, 2) the higher bodyweight with HbA1c < 7.0% at 54 weeks and/or 3) the specificity of the present cut-off point to predict long-term efficacy of liraglutide without any additional OADs. Since liraglutide has recently received new indications for co-administration with several OADs other than SU or insulin in Japan, β-cell function cut-off values for the efficacy of these combinations need to be determined.

Whether or not these cut-off values predict efficacy of other GLP-1 receptor agonists is not known. Recently, short- and long-acting GLP-1 receptor agonists have been shown to exert their glucose-lowering effects differently (Meier, 2012; Yabe & Seino, 2014). Long-acting agents ameliorate defects in islet function; short-acting agents inhibit gastric emptying. Consistently, the HbA1c-lowering effects of long-acting liraglutide but not those of the short-acting exenatide and lixisenatide depend on remaining β-cell function for their efficacy (Meier et al., 2011; Meier et al., 2013). It will be interesting to investigate association of remaining β-cell function with HbA1c-lowering effects of ultra-long-acting GLP-1 receptor agonists such as exenatide long-acting release, albiglutide, and duraglutide as well as those of dipeptidyl peptidase-4 inhibitors.

In the current study, liraglutide monotherapy and SU-combination therapy were analyzed in the same group due to limited sample size. Subjects who started liraglutide as monotherapy had longer duration in which to receive additional OADs including SU (Table 1); subjects who started liraglutide as SU-combination tended to have higher HbA1c at 54 weeks among those receiving no additional OAD for 54 weeks (data not shown). The percentage of SU-combination therapy was lower in patients receiving additional OAD (Table 1), suggesting that some patients received SU-combination therapy even though physicians in charge selected monotherapy. Future studies are required to analyze the association of remaining β-cell function with liraglutide monotherapy and SU-combinations separately, along with analysis in relation to liraglutide combinations with other OADs and insulin.

Remaining β-cell function did not affect weight reduction (Fig. 2). Liraglutide has been shown to suppress food intake and reduce bodyweight in mice lacking the GLP-1 receptor specifically in the peripheral nervous system but not in the central nervous system (Sisley et al., 2014). Functional MRI in individuals with type 2 diabetes revealed that the GLP-1 receptor agonist exenatide activates appetite- and reward-related brain areas (van Bloemendaal et al., 2014). Subcutaneously injected liraglutide in rats migrates and binds to GLP-1 receptors expressed in the arcuate nuclei of the hypothalamus, the brain region responsible for appetite control (Secher et al., 2014). These findings suggest direct actions of GLP-1 receptor agonist that reduce appetite and bodyweight independently of β-cell function.

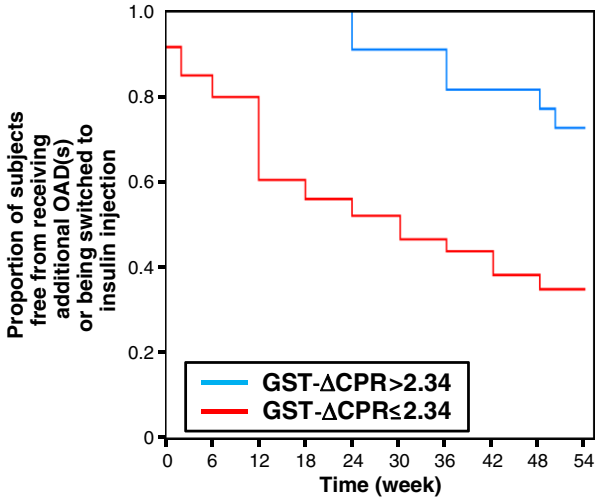


Fig. 4. Kaplan–Meier analysis for durability of initial therapy in patients with GST-ΔCPR >2.34 ng/mL (n = 22; sulfonylureas-combination n = 3 and monotherapy n = 19) and GST-ΔCPR ≤2.34 ng/mL (n = 93; sulfonylureas-combination n = 30 and monotherapy n = 63). Red, patients with GST-ΔCPR ≤2.34 ng/mL; Blue, those with GST-ΔCPR > 2.34 ng/mL The time before addition of an additional anti-diabetic drug or switch to insulin in order to maintain glycemic control was significantly different between the two groups (log-rank test, p = 0.0013).

The current study has limitations other than those described above, and caution is required. First, due to the nature of retrospective studies, there were no protocols for initiating liraglutide as monotherapy or SU-combination therapy, adding OADs or switching to insulin injections or reporting adverse events including hypoglycemia. Second, the number of subjects examined in this retrospective study is small. Furthermore, our analysis was conducted among subjects with GST data, who tend to have higher baseline HbA1c and to receive liraglutide initiation during their hospitalization. However, demographics other than baseline HbA1c were similar between subjects with and without GST data, suggesting that our findings might be generalized. Third, there were no inclusion or exclusion criteria for GST or liraglutide initiation due to the nature of retrospective studies. Although anti-diabetic drugs such as SU and insulin are known to affect the response to glucagon stimulation tests (Albareda et al., 2005), anti-diabetic drugs including insulin were not terminated until the night before GST, since hyperglycemia itself is known to affect GST (Nosari, Lepore, Maglio, Cortinovis, & Pagani, 1992). Fourth, higher bodyweight at 0 week suggests greater insulin resistance in patients achieving HbA1c <7.0% at 54 weeks, which might affect our cut-off value. Analysis with a larger sample size is required for adjustment for bodyweight and degree of insulin resistance in evaluation of the requirement for remaining β-cell function in regard to long-term glycemic control by liraglutide.

In conclusion, remaining β-cell function, assessed by GST-ΔCPR, plays a pivotal role in predicting long-term glycemic control by liraglutide. While further studies are needed for insulin- or other OAD-combination therapies, the cut-off value GST-ΔCPR 2.34 may be informative for classification of patients before initiation of liraglutide as monotherapy or SU-combination therapy.

Acknowledgement

The authors are deeply grateful to all past and present members of Center for Diabetes, Endocrinology and Metabolism; and Center for Metabolism and Clinical Nutrition who contributed to the work. The authors thank Professor Shimpei Fujimoto of Kochi University for discussion on glucagon stimulation test. The authors thank Michiko Yamane of Kansai Electric Power Hospital for secretarial assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jdiacomp.2015.07.020.

References

Albareda, M., Rigla, M., Rodriguez-Espinosa, J., Caballero, A., Chico, A., Cabezas, R., et al. (2005). Influence of exogenous insulin on C-peptide levels in subjects with type 2 diabetes. *Diabetes Research and Clinical Practice*, *68*(3), 202–206.

Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet*, *352*(9131)(1998)., 837–853.

U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: A progressive disease. U.K. Prospective Diabetes Study Group. *Diabetes*, *44*(11)(1999)., 1249–1258.

Best, J. D., Drury, P. L., Davis, T. M., Taskinen, M. R., Kesaniemi, Y. A., Scott, R., et al. (2012). Glycemic control over 5 years in 4,900 people with type 2 diabetes: Real-world diabetes therapy in a clinical trial cohort. *Diabetes Care*, *35*(5), 1165–1170.

DeFronzo, R. A. (1988). The triumvirate: β-Cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes*, *37*(6), 667–687.

Faber, O. K., & Binder, C. (1977). C-peptide response to glucagon. A test for the residual beta-cell function in diabetes mellitus. *Diabetes*, *26*(7), 605–610.

Fujita, Y., Kozawa, J., Iwahashi, H., Yoneda, S., Uno, S., Yoshikawa, A., et al. (2015). Increment of serum C-peptide measured by glucagon test closely correlates with human relative beta-cell area. *Endocrine Journal*, *62*(4), 329–337.

Funakoshi, S., Fujimoto, S., Hamasaki, A., Fujiwara, H., Fujita, Y., Ikeda, K., et al. (2011). Utility of indices using C-peptide levels for indication of insulin therapy to achieve good glycemic control in Japanese patientes with type 2 diaebtes. *Journal of Diabetes Investigation*, *2*(4), 297–303.

Gjessing, H. J., Matzen, L. E., Froland, A., & Faber, O. K. (1987). Correlations between fasting plasma C-peptide, glucagon-stimulated plasma C-peptide, and urinary C-peptide in insulin-treated diabetics. *Diabetes Care*, *10*(4), 487–490.

Greenbaum, C. J., Mandrup-Poulsen, T., McGee, P. F., Battelino, T., Haastert, B., Ludvigsson, J., et al. (2008). Type 1 Diabetes Trial Net Research G, et al. Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. *Diabetes Care*, *31*(10), 1966–1971.

Hendriksen, C., Faber, O. K., Drejer, J., & Binder, C. (1977). Prevalence of residual B-cell function in insulin-treated diabetics evaluated by the plasma C-etide response to intravenous glucagon. *Diabetologia*, *13*(6), 615–619.

Holman, R. R., Paul, S. K., Bethel, M. A., Matthews, D. R., & Neil, H. A. (2008). 10-year follow-up of intensive glucose control in type 2 diabetes. *The New England Journal of Medicine*, *359*(15), 1577–1589.

Iwao, T., Sakai, K., & Sata, M. (2013). Postprandial serum C-peptide is a useful parameter in the prediction of successful switching to liraglutide monotherapy from complex insulin therapy in Japanese patients with type 2 diabetes. *Journal of Diabetes and its Complications*, *27*(1), 87–91.

Iwata, M., Matsushita, Y., Fukuda, K., Wakura, T., Okabe, K., Koshimizu, Y., et al. (2014). Secretory units of islets in transplantation index is a useful predictor of insulin requirement in Japanese type 2 diabetic patients. *Journal of Diabetes Investigation*, *5*(5), 570–580.

Kajinuma, H., Kanazawa, Y., Sando, H., Hayashi, M., Kawazu, S., & Kosaka, K. (1979). Human plasma C-peptide immunoreactivity: Its correlation with immunoreactive insulin in diabetes, and chronic liver and renal diseases. *Endocrinologia Japonica*, *26*(1), 65–73.

Kaku, K., Rasmussen, M. F., Clauson, P., & Seino, Y. (2010). Improved glycaemic control with minimal hypoglycaemia and no weight change with the once-daily human GLP-1 analogue liraglutide as add-on to sulfonylurea in Japanese patients with type 2 diabetes. *Diabetes, Obesity & Metabolism*, *12*(4), 341–347.

Kaku, K., Rasmussen, M. F., Nishida, T., & Seino, Y. (2011). Fifty-two-week, randomized, multicenter trial to compare the safety and efficacy of the novel glucagon-like peptide-1 analog liraglutide vs glibenclamide in patients with type 2 diabetes. *Journal of Diabetes Investigation*, *2*(6), 441–447.

Kondo, Y., Satoh, S., Nagakura, J., Kimura, M., Nezu, U., & Terauchi, Y. (2013). Defining criteria for the introduction of liraglutide using the glucagon stimulation test in patients with type 2 diabetes. *Journal of Diabetes Investigation*, *4*(6), 571–575.

Kozawa, J., Inoue, K., Iwamoto, R., Kurashiki, Y., Okauchi, Y., Kashine, S., et al. (2012). Liraglutide is effective in type 2 diabetic patients with sustained endogenous insulin-secreting capacity. *Journal of Diabetes Investigation*, *3*(3), 294–297.

Lapolla, A., Frison, V., Bettio, M., Pos, M. D., Rocchini, P., Panebianco, G., et al. (2015). Correlation between baseline characteristics and clinical outcomes in a large population of diabetes patients treated with liraglutide in a real-world setting in Italy. *Clinical Therapeutics*, *37*(3), 574–584.

Matsuda, A., Kamata, I., Iwamoto, Y., Sakamoto, Y., & Kuzuya, T. (1985). A comparison of serum C-peptide response to intravenous glucagon, and urine C-peptide, as indexes of insulin dependence. *Diabetes Research and Clinical Practice*, *1*(3), 161–167.

Meier, J. J. (2012). GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nature Reviews. Endocrinology*, *8*(12), 728–742.

Meier, J. J., Vilsbøll, T., Donsmark, M., Hartvig, H., & Nauck, M. A. (2011). Greater glycaemic control is achieved with the once-daily human GLP-1 analogue liraglutide vs comparators across the continuum of estimated beta cell mass. *Diabetologia*, *54*(Supplment 1), S322.

Meier, J. J., Yabe, D., Wang, E., Lin, J., Rosenstock, J., & Ahren, B. (2013). Efficacy of lixisenatide in patients with different levels of beta cell function as assessed by C-peptide/glucose ratio. *Diabetologia*, *56*(Supplement1), S357.

Nosari, I., Lepore, G., Maglio, M. L., Cortinovis, F., & Pagani, G. (1992). The effect of various blood glucose levels on post-glucagon C-peptide secretion in type 2 (non insulin-dependent) diabetes. *Journal of Endocrinological Investigation*, *15*(2), 143–146.

Ponzani, P. (2013). Long-term effectiveness and safety of liraglutide in clinical practice. *Minerva Endocrinologica*, *38*(1), 103–112.

Retnakaran, R., Kramer, C. K., Choi, H., Swaminathan, B., & Zinman, B. (2014). Liraglutide and the preservation of pancreatic beta-cell function in early type 2 diabetes: The LIBRA trial. *Diabetes Care*, *37*(12), 3270–3278.

Saisho, Y., Kou, K., Tanaka, K., Abe, T., Kurosawa, H., Shimada, A., et al. (2011). Postprandial serum C-peptide to plasma glucose ratio as a predictor of subsequent insulin treatment in patients with type 2 diabetes. *Endocrine Journal*, *58*(4), 315–322.

Secher, A., Jelsing, J., Baquero, A. F., Hecksher-Sorensen, J., Cowley, M. A., Dalboge, L. S., et al. (2014). The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss. *The Journal of Clinical Investigation*, *124*(10), 4473–4488.

Seino, Y., Nanjo, K., Tajima, N., Kadowaki, T., Kashiwagi, A., Araki, E., et al. (2010). Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus. *Journal of Diabetes Investigation*, *1*(5), 212–228.

Seino, Y., Rasmussen, M. F., Clauson, P., & Kaku, K. (2012). The once-daily human glucagon-like peptide-1 analog, liraglutide, improves beta-cell function in Japanese patients with type 2 diabetes. *Journal of Diabetes Investigation*, *3*(4), 388–395.

Seino, Y., Rasmussen, M. F., Nishida, T., & Kaku, K. (2010). Efficacy and safety of the once-daily human GLP-1 analogue, liraglutide, vs glibenclamide monotherapy in Japanese patients with type 2 diabetes. *Current Medical Research and Opinion*, *26*(5), 1013–1022.

Seino, Y., Rasmussen, M. F., Nishida, T., & Kaku, K. (2011). Glucagon-like peptide-1 analog liraglutide in combination with sulfonylurea safely improves blood glucose measures vs sulfonylurea monotherapy in Japanese patients with type 2 diabetes: Results of a 52-week, randomized, multicenter trial. *Journal of Diabetes Investigation*, *2*(4), 280–286.

Shao, Y., Yuan, G., Feng, Y., Zhang, J., & Guo, X. (2014). Early liraglutide treatment is better in glucose control, beta-cell function improvement and mass preservation in db/db mice. *Peptides*, *52*, 134–142.

Sisley, S., Gutierrez-Aguilar, R., Scott, M., D'Alessio, D. A., Sandoval, D. A., & Seeley, R. J. (2014). Neuronal GLP1R mediates liraglutide's anorectic but not glucose-lowering effect. *The Journal of Clinical Investigation*, *124*(6), 2456–2463.

Stumvoll, M., Goldstein, B. J., & van Haeften, T. W. (2005). Type 2 diabetes: Principles of pathogenesis and therapy. *Lancet*, *365*(9467), 1333–1346.

Toyoda, M., Yokoyama, H., Abe, K., Nakamura, S., & Suzuki, D. (2014). Predictors of response to liraglutide in Japanese type 2 diabetes. *Diabetes Research and Clinical Practice*, *106*(3), 451–457.

Usui, R., Yabe, D., Kuwata, H., Fujiwara, S., Watanabe, K., Hyo, T., et al. (2013). Retrospective analysis of safety and efficacy of insulin-to-liraglutide switch in Japanese type 2 diabetes: A caution against inappropriate use in patients with reduced beta cell function. *Journal of Diabetes Investigation*, *4*(6), 585–594.

van Bloemendaal, L., RG, I. J., Ten Kulve, J. S., Barkhof, F., Konrad, R. J., Drent, M. L., et al. (2014). GLP-1 receptor activation modulates appetite- and reward-related brain areas in humans. *Diabetes*, *63*(12), 4186–4196.

Vilsboll, T., Brock, B., Perrild, H., Levin, K., Lervang, H. H., Kolendorf, K., et al. (2008). Liraglutide, a once-daily human GLP-1 analogue, improves pancreatic B-cell function and arginine-stimulated insulin secretion during hyperglycaemia in patients with type 2 diabetes mellitus. *Diabetic Medicine*, *25*(2), 152–156.

Yabe, D., Kuwata, H., Usui, R., Kurose, T., & Seino, Y. (2015). Glucagon-like peptide-1 receptor agonist therapeutics for total diabetes management: Assessment of composite end-points. *Current Medical Research and Opinion*, 1–13.

Yabe, D., & Seino, Y. (2011). Liraglutide in adults with type 2 diabetes: Global perspective on safety, efficacy and patient preference. *Clinical Medicine Insights: Endocrinology and, Diabetes*, *4*, 47–62.

Yabe, D., & Seino, Y. (2014). Defining the role of GLP-1 receptor agonists for individualized treatment of type 2 diabetes. *Expert Review of Endocrinology and Metabolism*, *9*(6), 659–670.

Yabe, D., Seino, Y., Fukushima, M., & Seino, S. (2015). β Cell dysfunction versus insulin resistance in the pathogenesis of type 2 diabetes in East Asians. *Current Diabetes Reports*, *15*(6), 602–611.

Yamada, Y., Fukuda, K., Fujimoto, S., Hosokawa, M., Tsukiyama, K., Nagashima, K., et al. (2006). SUIT, secretory units of islets in transplantation: An index for therapeutic management of islet transplanted patients and its application to type 2 diabetes. *Diabetes Research and Clinical Practice*, *74*(3), 222–226.